UCRL-JC-120360 ABS

CHROMOSOMAL ABNORMALITIES DETECTED IN ZYGOTES AND 4-DAY EMBRYOS OF MICE USING MULTI-PROBE FISH

X. Lowe[1], F. Marchetti[1], T. Ahlborn[1,2], J. Bishop[3], N. Titenko-Holland[2], M. T. Smith[2], and A. J. Wyrobek[1]

[1]Biology and Biotechnology Research Program, Lawrence Livermore National Laboratory, Livermore, CA; [2]School of Public Health, University of California, Berkeley, CA; [3]National Institute of Environmental Health Sciences, RTP, NC

Pre-implantation is a sensitive period of early mammalian development, and embryos carrying cytogenetic defects are generally less viable and more susceptible to morphological abnormalities before and after birth. We are developing new methods to detect molecular cytogenetic defects near both extremes of the pre-implantation period (i.e., zygote and 4 day post conception). These methods are being used to characterize the induction and persistence of cytogenetic damage during early development and the fate of embryos carrying cytogenetic defects. First cleavage metaphase spreads were prepared from one-cell zygotes isolated by a mass harvest procedure. Metaphase spreads were labeled using multi-color fluorescence in situ hybridization (FISH) with various combinations of high-complexity chromosome-specific painting probes. The zygote FISH methods are being applied to determine the levels of translocations, dicentrics and aneuploidies inherited via either of the parental germ cells. The cells of the 4-day blastocysts were prepared for interphase FISH analysis by uterine flushing, cell spreading in hypotonic solution, and sequential fixation. Micronuclei are being detected using DAPI and characterized with pan-centromeric DNA probes. Ploidy is being assessed using DNA probes for both sex chromosomes. These methods have applications in studies of genetic factors affecting embryo susceptibility, embryo toxicology, and for determining the developmental consequences of genetic damage transmitted via maternal or paternal germ cells.

[Work was performed under the auspices of the U.S. DOE by the Lawrence Livermore National Laboratory under contract W-7405-ENG-48 with support from NIEHS Y01-ES-10203-00 and Program for Molecular Cytology, UC San Francisco].